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Qualitative differences between naïve and memory T cells

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INTRODUCTION

Mature T cells are produced in the thymus and released into the bloodstream in low numbers. These cells are considered to be immunologically naïve until such time as they encounter MHC-peptide complexes for which their T-cell receptors (TCR) have high affinity. Recognition of antigen in appropriate form, i.e. in association with costimulatory signals on the surface of professional antigen-presenting cells (APCs), leads to extensive T-cell proliferation and differentiation into effector cells. Once the infection has been cleared, it is no longer of benefit to the host to maintain high numbers of effector cells and most of the activated T cells die by apoptosis. However, a proportion of these cells survive, leaving the frequency of cells specific for the priming antigen much higher among memory T cells than that which existed among naïve T cells. This difference in frequency makes a major contribution to the nature of the secondary response, which is typically faster and of greater magnitude than the primary response. In addition, T cells may also carry a true 'memory' of a prior response to antigen, exhibiting differences from naïve T cells at the single cell level. Here we provide a brief overview of the qualitative differences that have been reported to exist between naïve and memory T cells and evidence that memory T cells themselves are functionally heterogeneous.

PHENOTYPIC DIFFERENCES BETWEEN NAÏVE AND MEMORY T CELLS

The supposition that naïve and memory T cells can be distinguished phenotypically is based on the notion that memory T cells retain a permanent imprint of having responded to antigen. Precise identification of memory T cells, however, remains problematic. Unlike B cells, T cells do not appear to mutate their antigen receptor genes during the course of an immune response. Furthermore, discrimination between effector and memory T cells is accomplished on the basis of rather nebulous criteria; memory T cells are considered to differ from effector T cells by their continued survival after the acute immune response has died down and by being in a lower state of activation. As discussed further below, these distinctions are becoming increasingly blurred.

Despite these difficulties, a number of phenotypic differences between naïve and memory T cells have been noted. Most of these are changes that arise during initial T-cell activation and appear to persist in memory cells. Especially prominent are differences in the cell surface expression of adhesion molecules between naïve and memory T cells. Thus, compared to naïve T cells, memory T cells have been reported to express higher levels of β1 (CD29, CD49d and CD49e) and β2 (CD11a, CD11b and CD18) integrins, CD2, CD44, CD54 and CD58.[1–11] Increased expression of adhesion molecules on recently activated T cells reflects the requirements for effector T cells to enter peripheral tissues at sites of inflammation and interact with target cells, and may similarly affect the function of some memory T cells (see below).

The expression of other molecules involved in lymphocyte migration also differs between naïve and memory T cells. Of particular interest are differences in the expression of two key molecules required for the entry of T cells into lymph nodes through high endothelial venules (HEVs): CD62L and CCR7. CD62L binds to vascular addressins expressed on HEVs and is responsible for the initial stage of adherence of blood-borne T cells to HEVs,[12] while the CCR7 chemokine receptor controls responsiveness to chemokines expressed in HEVs at sites of lymphocyte entry.[13] Whereas naïve T cells are uniform in expressing high levels of both molecules, some memory cells lose expression of CD62L and/or CCR7.[14–17] However, memory T cells may express receptors for chemokines that direct them to inflammatory sites and for molecules involved in homing to peripheral tissues, such as the cutaneous lymphocyte antigen (CLA) which is involved in lymphocyte migration to skin.[17]

Other cell surface molecules that have been reported to distinguish between naïve and memory T cells include the IL-2R β-chain (CD122),[18,19] Ly-6C[19–21] and the common leukocyte antigen (CD45).[4,6,14,22–28] CD122 is a component of both the IL-2R and the IL-15R and may play a role in the maintenance of memory T cells (see below),[29] while

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Ly-6C is a low-molecular-weight (MW) glycosylphophatidylinositol-anchored molecule that has been proposed to participate in intercellular adhesion;[30] both CD122 and Ly-6C are expressed at high levels on $CD8^+$ memory T cells in the mouse. For CD45, which is a tyrosine phosphatase that regulates signalling through antigen receptors and cytokine receptors,[31,32] it is the form of the molecule that differs between naïve and memory cells, rather than the level of expression. Multiple isoforms of CD45 are generated by differential splicing of three extracellular exons (A, B and C). These restricted (R) isoforms can be detected specifically with mAb directed against the variably spliced exons. Naïve T cells express the highest MW isoform, containing all three of these exons (commonly referred to as CD45RA in humans). During the course of T-cell activation, T cells switch to expressing lower-MW isoforms; in humans at least, activated T cells express the isoform of CD45 lacking all three variably spliced exons (defined as CD45R0). In many different species, expression of low-MW isoforms of CD45 is retained on memory cells.

Since many of the phenotypic properties associated with memory T cells are in fact acquired soon after activation, these markers cannot be used on their own to discriminate between recently activated cells and memory cells. This distinction can be aided to a certain extent by combining these phenotypic markers with other criteria to exclude T cells that are actively responding to antigen. For example, it is generally assumed that memory T cells do not have a blasted morphology and do not express transient markers of activation such as CD69. Furthermore, differences in cell surface glycosylation have been reported to exist between effector and memory $CD8^+$ T cells in mice. Specifically, memory cells have a higher degree of sialylation on 1 O-glycans and express lower levels of 2 O-glycans than effector cells;[33–36] this difference can be detected using an antibody that binds specifically to CD43 only when this molecule has been modified by 2 O-glycans.[37] However, in using signs of recent activation as exclusion criteria for memory T cells, it must be borne in mind that even long-term memory cells appear to be more metabolically active than naïve T cells.[38] As discussed below, memory T cells undergo periodic rounds of cell division even in the complete absence of antigen. Therefore, markers of recent activation cannot be used definitively to distinguish between effector and memory T cells.

Although memory T cells are enriched amongst cells expressing the surface markers discussed above, it is also clear that memory cells exhibit substantial phenotypic heterogeneity. This issue has received considerable attention in recent years, with interest stemming largely from a report that $CD45R0^+$ T cells in human blood can be divided into $CD62L^+CCR7^+$ and $CD62L^-CCR7^-$ subpopulations.[17] These cells have been termed 'central memory' and 'effector memory' cells, respectively, based on their expression of lymph node homing molecules and their functional properties (see below). In addition to CD62L and CCR7, memory T cells may express other markers associated with naïve T cells, such as high-MW isoforms of CD45.[16,17,39–46]

In some instances, it is evident that expression of a 'naïve' phenotype by primed cells represents phenotypic reversion. This has been shown to be the case for CD62L, CCR7 and high-MW isoforms of CD45, each of which can be re-expressed by cells that were formerly negative for these markers.[39,41,47–52] Phenotypic reversion occurs at different rates in different species and also differs for CD4 vs. CD8 cells. For example, rat $CD45RC^-$ (memory-phenotype) $CD4^+$ T cells re-express CD45RC within 1 week when transferred to secondary recipients in the absence of antigen;[39,41] the rapidity with which this reversion takes place suggests that the $CD45RC^-$ phenotype in the rat is a marker of recent activation rather than memory. Conversely, $CD4^+$ memory T cells in mice can maintain a $CD45RB^{low}$ phenotype for at least 10 weeks in the absence of antigen, although $CD8^+$ T cells re-express CD45RB soon after activation.[53]

Phenotypic reversion is presumed to reflect a 'cooling down' of activated cells; lack of contact with antigen results in a return to a resting state and the loss of expression of activation molecules. By corollary, retention of memory markers may be indicative of periodic contact with persisting antigen. However, some phenotypic markers, particularly the expression of high levels of CD44 on mouse memory T cells, appear to be retained long-term in the complete absence of antigen.[54,55] In addition, mechanisms other than reversion may account for some of the phenotypic heterogeneity observed amongst memory T cells. In this respect, it is notable that some $CD45RA^+$ $CD8^+$ T cells in human peripheral blood exhibit the properties of activated effector cells.[11] These cells, which also express low levels of CD28 and CD27, appear to arise from chronic antigenic stimulation.[56,57] Likewise, some primed $CD45RA^+$ $CD4^+$ T cells can be found under conditions of chronic antigen exposure.[45]

Whether the $CD45RA^+$ cells observed in these studies have in fact re-expressed this molecule is unclear. Another possibility is that some cells may retain expression of CD45RA under certain conditions of activation. This is worth considering in view of data showing that mouse $CD8^+$ T cells can differentiate directly into cells with the properties of central memory cells following antigenic stimulation *in vitro*.[58,59] In these studies, brief exposure of $CD8^+$ T cells to antigen followed by culture in IL-15 or low doses of IL-2 generated T cells that retained expression of CD62L and CCR7 and which lacked overt effector activity. By contrast, cells exposed to high concentrations of IL-2 after antigenic stimulation lost expression of CD62L and CCR7 and differentiated into effector cells. Therefore, acquisition of all of the markers typically associated with T-cell activation is not an inevitable consequence of antigenic stimulation.

Also complicating the use of phenotypic markers to distinguish between naïve and memory T cells is the fact that naïve T cells can acquire markers of memory cells in the absence of overt antigenic stimulation. This has been demonstrated to occur when small numbers of naïve cells are adoptively transferred into lymphopenic recipients.[60–68] Under these conditions, naïve T cells proliferate slowly

(so-called homeostatic proliferation), up-regulate expression of activation/memory markers and exhibit effector activity. Notably, this response is driven not by specific antigen but by self-peptide-MHC complexes in combination with cytokines.[60,61,64,69–71] The contribution of cells generated by this process to the pool of memory-phenotype T cells in normal mice is unclear. However, the fact that very few memory-phenotype T cells are observed in germ-free mice argues that most memory-phenotype T cells are derived from antigenic stimulation.[27] Nevertheless, it remains possible that the phenotypic conversion associated with homeostatic proliferation may make a significant contribution to the pool of memory-phenotype T cells under conditions of lymphopenia.

NAÏVE AND MEMORY T CELLS EXHIBIT QUALITATIVELY DIFFERENT RESPONSES TO ANTIGEN

It has been evident for many years that memory-phenotype T cells respond to antigen in a qualitatively different way from naïve-phenotype T cells.[2,27,72–81] In recent years, experiments employing TCR transgenic T cells have provided strong evidence that this is also true for bona fide naïve and memory cells. Differences are manifest at two levels.

Firstly, memory T cells appear to have less stringent requirements for activation than naïve T cells. This may include an ability to respond to lower concentrations of antigen than naïve T cells,[21,82–85] although some investigators have failed to find any difference between naïve and memory T cells in their sensitivity to antigen.[86–88] In addition, memory T cells are less dependent on costimulatory signals than naïve T cells, and do not require as long a duration of antigenic stimulation. Each of these factors may contribute to the fact that a wider range of cells can act as APCs for memory T cells compared to naïve T cells. Thus, while activation of naïve T cells is strictly dependent on antigen presentation by dendritic cells (DCs),[89] memory T cells respond to antigen presented on other APCs, including resting B cells.[90]

Secondly, once they have been activated, the response characteristics of naïve and memory T cells also differ. For example, there is some evidence that memory T cells proliferate faster and reach higher numbers *in vivo* than naïve T cells following antigenic stimulation.[91–93] However, this issue remains contentious, as these differences have not been detected in other studies.[88] Conversely, it is a general finding that memory T cells display effector functions sooner after activation than naïve T cells. This includes the expression of cytolytic activity (for $CD8^+$ T cells) and the secretion of cytokines other than IL-2.

Some clues as to the biochemical basis of the altered responsiveness of memory T cells have been uncovered. For instance, the rapid expression of effector cytokines by memory T cells may be linked to the modulation of chromatin structure or demethylation of promoters for cytokine genes; this has been shown to occur following initial T-cell activation and is inherited by daughter cells.[94,95] Indeed, some memory T cells have been shown to express mRNA for effector cytokines prior to secondary stimulation.[92,93] In addition, non-dividing memory T cells possess a higher content of both RNA and protein than naïve T cells, suggesting that memory cells may be resting in the G_1 rather than the G_0 phase of the cell cycle.[38,93] This might allow for faster entry into S phase and initiation of DNA replication by memory T cells following TCR triggering. Furthermore, several differences between naïve and memory T cells have been noted that could affect signalling pathways within these cells. Specifically, naïve and memory T cells have been shown to differ with respect to phosphorylation and association of signalling components of the TCR/CD3 complex,[96] expression of the linker/adapter molecule SLP−76[97] and association of CD45 with the TCR or CD4.[98,99]

Another factor that could contribute to the altered properties of memory T cells is an enhanced ability to interact with APCs compared to naïve T cells. Given their increased expression of various adhesion molecules (see above), it seems likely that memory cells will form higher avidity interactions with other cells; this could contribute to the wider range of APCs utilized by memory vs. naïve T cells. Furthermore, there is evidence that T cells are selectively recruited on the basis of expressing TCRs with higher affinity for antigen during the course of an immune response.[100–110] This is a result of the loss of cells expressing TCRs with the fastest dissociation rates for peptide-MHC binding.[104,106] Since TCR genes do not undergo somatic hypermutation, this process is not directly analogous to the affinity maturation involved in the generation of memory B cells, and results in relatively modest increases in affinity. Nevertheless, the data suggest that memory T cells may represent a selected cell population having a higher average affinity for antigen than the starting pool of antigen-responsive naïve cells.

As discussed above, memory T cells are phenotypically heterogeneous. While the extent to which this heterogeneity also applies to the functional properties of memory cells remains to be fully investigated, it is evident that central memory cells and effector memory cells exhibit clear differences in their response to antigen. Thus, a direct comparison of these two subpopulations showed that $CD4^+$ $CD45R0^+$ $CCR7^-$ effector memory cells secrete a broad range of cytokines (IL-2, IFN-γ, IL-4 and IL-5) within 24 hr of stimulation through the TCR, while $CD4^+$ $CD45R0^+$ $CCR7^+$ central memory cells secrete only IL-2.[17] In addition, effector memory cells were also able to proliferate in response to lower concentrations of anti-CD3 antibodies than central memory cells, although the latter were still more responsive than naïve T cells. Therefore, memory T cells are in fact functionally heterogeneous, and any general distinction between the functional properties of naïve and memory T cells is clearly an oversimplification. This heterogeneity may partially account for discrepancies in the literature regarding differences between naïve and memory cells.

MIGRATION OF NAÏVE AND MEMORY T CELLS

Lymphocyte migration is controlled by the combination of adhesion molecules and chemokine receptors expressed by lymphocytes. Since the expression of these molecules differs between naïve and memory T cells, it is not surprising that these cells have different migratory properties.

Naïve T cells exhibit a restricted pattern of migration, in which they move continuously between the secondary lymphoid organs (spleen, lymph nodes and Peyer's patches) via blood and lymph; transit through individual organs takes about 12–18 hr.[111] The expression of CD62L and CCR7 by naïve T cells plays a key role in establishing this recirculation pattern. Thus, entry of naïve T cells into lymph nodes and Peyer's patches occurs at HEVs in a CD62L- and CCR7-dependent manner (see above). In addition, although initial entry of lymphocytes into the spleen is a passive process, with T cells being deposited from the blood into the marginal zone, migration of cells into the T-cell zones of the spleen may also require expression of CCR7.[112] However, naïve T cells lack homing receptors for peripheral tissues and chemokine receptors for inflammatory cytokines and are therefore unable to enter non-lymphoid tissues.

By contrast, some memory T cells express chemokine and adhesion receptors that enable them to extravasate into non-lymphoid tissues.[17] Migration into peripheral tissues was originally demonstrated for memory-phenotype T cells in sheep and subsequently demonstrated for antigen-specific memory T cells in mice.[6,59,113–116] However, as discussed above, memory T cells are heterogeneous with respect to the expression of homing molecules and likewise exhibit heterogeneity in migration. Central memory cells, which express CD62L and CCR7, are able to enter lymph nodes via HEVs and exhibit a pattern of migration that is similar to that of naïve T cells. Conversely, effector memory cells lack CD62L and CCR7 but express homing receptors that allow them to enter non-lymphoid tissues; these cells may reach lymph nodes via afferent lymph rather than through HEVs.[6] In addition, subpopulations of memory T cells exhibit a preference for migration to particular tissues, for example gut vs. skin, on the basis of the particular homing molecules expressed.[113,117–122] This predilection for specific tissues may be dictated by the local environment in which initial T-cell priming occurs.[121–123]

The migration pathways favoured by naïve and memory T cells are linked to their activation requirements and functional properties. As described above, activation of naïve T cells requires antigen presentation on mature DCs. These APCs are found in the T-cell zones of secondary lymphoid organs; DCs move into these areas and present antigen following the uptake of pathogens in peripheral sites.[124] Hence, secondary lymphoid organs serve as predetermined meeting places for naïve T cells and DCs that maximize the chance of low-frequency T cells encountering their specific antigen. Continuous movement of naïve T cells between different lymphoid organs allows these cells to scan the surface of DCs derived from all parts of the body. In addition, the environment of the organised lymphoid tissues is ideal for expansion of activated T cells and their differentiation into effectors.

Conversely, activation of memory T cells can occur outside the optimized conditions of the secondary lymphoid organs, owing both to the increased frequency of antigen-specific cells among memory cells and to their ability to become activated by a wider range of APCs. As a consequence, effector memory T cells can efficiently perform a surveillance function at potential sites of pathogen invasion and provide a rapid response to re-infection. In contrast, central memory T cells, which appear to migrate in a similar manner to naïve T cells, probably respond to antigen in secondary lymphoid organs. Whether central memory cells actually require antigen presentation on DCs in order to undergo activation and differentiation into effector cells is unclear (see above). Nevertheless, it seems likely that a much greater degree of expansion will occur following antigenic stimulation in lymphoid organs. Therefore, central and effector memory T cells may provide complementary functions upon secondary infection, with effector memory cells providing an immediate local response and central memory cells rapidly generating large numbers of effectors.

LIFESPAN OF NAÏVE VS. MEMORY T CELLS

In general, cell populations can be maintained at constant numbers by two mechanisms: (1) survival of long-lived cells that do not divide, and (2) proliferation of shorter lived cells, which is balanced by the same rate of cell death (turnover). Under non-disease conditions, the overall rate of T-cell turnover is relatively slow, indicating that these cells can remain in interphase for long periods of time. This was shown initially in rodents, where T-cell proliferation *in vivo* was measured by infusion of a labelled DNA precursor, ^{3}H-thymidine.[125–129] From this type of study, the turnover time for T cells in the thoracic duct of mice was calculated to be of the order of 4–6 months.[127] More recent studies using ^{2}H-glucose as a DNA precursor have shown that human T cells also turn over slowly.[130] Here, the mean intermitotic times for $CD4^{+}$ and $CD8^{+}$ T cells in peripheral blood were estimated to be 87 and 77 days, respectively.

Studies in a number of different species, including mice,[131] sheep,[6] rhesus macaques[132] and humans,[133,134] have shown that memory-phenotype T cells turn over at a faster rate than naïve-phenotype T cells. On average, the rate of incorporation of labelled DNA precursors is approximately 5 times faster for memory-phenotype cells than naïve-phenotype cells. Extrapolation of these data directly to naïve and memory T cells is complicated by the limitations of using phenotypic markers to identify these cells (see above). However, it is notable that studies of cell populations that can be designated as memory T cells with high confidence – cells analysed at extended periods after immunization, using TCR transgenics or MHC tetramers to identify antigen-specific cells – have shown that these cells exhibit similar rates of division *in vivo* to memory-phenotype T cells in normal mice.[55,135,136] The implication therefore is that memory T cells are maintained by

intermittent cell division while naïve T cells persist as relatively quiescent, non-dividing cells.

Although memory-phenotype T cells exhibit rapid turnover overall, some memory T cells appear to remain in a non-dividing state for long periods of time. This is evident from the results of DNA labelling studies, which show considerable heterogeneity in the rates at which cells incorporate label during the infusion phase or lose label after the termination of treatment.[131,134] This is not surprising in view of the evidence cited above for heterogeneity amongst memory T cells. At present, it is unknown whether long- and short-lived memory T cells correspond to subpopulations of cells that can be distinguished phenotypically (e.g. central vs. effector memory T cells).

FACTORS CONTROLLING THE MAINTENANCE OF NAÏVE AND MEMORY T CELLS

In addition to exhibiting distinct kinetic behaviour, naïve and memory T cells are also dependent on different factors for maintaining their survival. Two main stimuli have been studied in this regard: (1) MHC-peptide, and (2) cytokines.

MHC-peptide

The issue of whether long-term survival of naïve T cells is dependent on contact with self-MHC-peptide ligands has been studied extensively.[55,137–145] Because MHC expression in the thymus is essential for the initial development of T cells, investigating the role of peripheral MHC in T-cell survival requires either adoptive transfer of mature T cells into MHC-deficient hosts or selective and transient expression of MHC in the thymus of such mice. In general, these studies have shown that the lifespan of naïve $CD4^+$ and $CD8^+$ T cells is shortened in the absence of MHC class II or class I, respectively. However, it is notable that naïve $CD4^+$ T cells can still survive for considerable periods of time in the absence of MHC class II. Thus, the half-life of naïve $CD4^+$ cells in the absence of MHC class II has been estimated to be approximately 3–4 weeks by some investigators,[140,145] while others have reported that naïve CD4 cells can survive equally well in control and MHC class II-deficient hosts for the first 4^{144} or 8^{143} weeks after transfer. These results imply that naïve CD4 cells require only very infrequent contact with MHC in order to survive.

The nature of the MHC-ligands involved in transmitting survival signals to naïve T cells is unknown. For homeostatic proliferation (see above), T cells respond to self-MHC in a peptide-specific manner, with the peptides involved being at least partially related to those involved in T-cell positive selection in the thymus.[144,146–148] Therefore, one possibility is that the same MHC-peptide ligands that stimulate proliferation in lymphopenic animals trigger survival under normal conditions. Conversely, survival of naïve T cells may depend only upon recognition of MHC and be independent of the bound peptide.

There has been extensive debate surrounding the role of persisting antigen in the maintenance of T-cell memory. While it remains a contentious issue whether periodic contact with antigen is required to maintain protective immunity,[149–153] it is now generally accepted that the survival of memory T cells is antigen-independent. Thus, numerous studies in mice have shown that both $CD4^+$ and $CD8^+$ memory T cells of defined specificity survive indefinitely following adoptive transfer into antigen-free hosts.[54,55,91–93,154–157] Furthermore, long-term survival also applies to $CD4^+$ and $CD8^+$ memory T cells transferred to MHC class II- and MHC class I-deficient mice, respectively.[55,157–159] Therefore, unlike naïve T cells, memory T cells do not appear to depend on interactions with MHC for survival. Notably, memory T cells also continue to proliferate in MHC-deficient hosts.[55,141,157,159] For $CD8^+$ memory T cells, proliferation occurs at similar rates after transfer to MHC class $I^{+/+}$ or MHC class $I^{-/-}$ hosts, implying that turnover, like survival is regulated by signals independent of TCR triggering.[55]

By contrast, it is less clear whether the functional properties of memory T cells are also independent of contact with MHC. For some properties of memory T cells, this does appear to be the case. Thus, $CD4^+$ and $CD8^+$ memory T cells retain a $CD44^{hi}$ phenotype and the capacity for rapid secretion of IFN-γ after transfer to MHC-deficient mice.[55,159] However, it has been reported that CD4 memory cells parked in MHC class II-deficient hosts lose other functional characteristics ascribed to memory cells, namely the ability to respond to antigen presented by non-professional APCs such as B cells and a relative independence from costimulation.[159] Whether a similar requirement for contact with MHC exists for CD8 memory cells remains to be investigated.

Cytokines

The main cytokine that has been implicated in maintaining the survival of naïve T cells is IL-7. In fact, IL-7 seems to be essential for naïve T cell survival, as these cells disappear rapidly following adoptive transfer to IL-$7^{-/-}$ recipients or in normal mice treated with anti-IL-7R antibodies; in addition, IL-$7R^{-/-}$ naïve T cells fail to survive in normal hosts.[70,160,161] Although other cytokines such as IL-4, IL-6 and IL-15 have been shown to rescue naïve T cells from apoptosis *in vitro*[162,163] (M. Berard and D. F. Tough, unpublished data), these cytokines do not appear to be required for the survival of naïve T cells *in vivo* and cannot compensate for the absence of IL-7.[161] In addition to promoting the survival of naïve T cells in normal mice, IL-7 also seems to be required for homeostatic proliferation of naïve T cells under lymphopenic conditions.[70,160] Thus, maintenance of naïve T cells appears to be strictly dependent on contact with both self-MHC-peptide ligands and IL-7.

Evidence that cytokines play a role in the maintenance of memory T cells came initially from studies showing that injection of either type I IFN (IFN-α/β) or inducers of IFN-α/β stimulated TCR-independent proliferation of memory-phenotype ($CD44^{hi}$) $CD8^+$ T cells *in vivo*[164,165] Subsequently, a number of other cytokines, including IL-12, IL-15, IL-18 and IFN-γ, were found to have similar

effects.[18,166] Notably, enhanced proliferation was restricted to $CD44^{hi}$ $CD8^+$ T cells following injection of each of these cytokines; little or no cell division was induced amongst naïve phenotype T cells. Of the cytokines shown to promote proliferation of memory-phenotype $CD8^+$ T cells *in vivo*, however, only IL-15 was able to stimulate these cells to divide when added to purified T cells *in vitro*. Here, the selective proliferation of memory-phenotype $CD8^+$ T cells in response to IL-15 was associated with much higher expression of the IL-15R β-chain (CD122) on $CD44^{hi}$ vs. $CD44^{lo}$ $CD8^+$ T cells.[18] Since IFN-α/β, IFN-γ, IL-12 and IL-18 are all capable of up-regulating IL-15 expression by APCs (the latter two through induction of IFN-γ), it has been proposed that IL-15 acts as a final common effector molecule mediating the effects on $CD44^{hi}$ $CD8^+$ T cell proliferation.[18,166]

Based on the observation that injection of IL-15 or induction of IL-15 expression *in vivo* results in enhanced proliferation of memory-phenotype $CD8^+$ T cells, it was hypothesized that the high proliferation of these cells in normal mice might be attributable to background production of IL-15. In support of this idea, injection of anti-CD122 antibodies into mice was shown to strongly reduce the proliferation of $CD44^{hi}$ $CD8^+$ T cells.[167] Although this antibody can block signalling through both the IL-15R and the IL-2R, the inhibitory effects appeared to result from blocking IL-15, since injection of anti-IL-2 + anti-IL-2Rα actually enhanced $CD44^{hi}$ $CD8^+$ T-cell proliferation. The conclusion from this study was that IL-15 and IL-2 had opposing effects on the proliferation of memory-phenotype CD8 T cells *in vivo*, with IL-15 enhancing and IL-2 inhibiting cell division.

Strikingly, the number of memory-phenotype $CD8^+$ T cells is markedly reduced in both IL-15- and IL-15Rα-deficient mice,[168,169] and increased in transgenic mice over-expressing IL-15.[170,171] Thus, IL-15 appears to play an important role in regulating the number of $CD44^{hi}$ $CD8^+$ T cells found in normal mice; definitive evidence of its importance for antigen-specific CD8 memory T cells awaits further study in infection/immunization models. Exactly how IL-15 controls the maintenance of $CD44^{hi}$ $CD8^+$ T cells remains unclear. The simplest idea is that IL-15-induced proliferation is necessary to counter a rapid rate of death; increases or decreases in the amount of IL-15 tip the balance towards more or less accumulation of $CD44^{hi}$ $CD8^+$ T cells, respectively. However, the fact that a considerable proportion of memory-phenotype $CD8^+$ T cells can persist for long periods of time in a non-dividing state is inconsistent with the idea that all memory cells are intrinsically short-lived.[131] Therefore, an alternative possibility is that IL-15 is able to promote survival as well as proliferation of $CD44^{hi}$ $CD8^+$ T cells. In this respect, it is notable that IL-15 induces up-regulated expression of the pro-survival factor Bcl-2 in $CD8^+$ T cells.[171]

In contrast to its effects on $CD44^{hi}$ $CD8^+$ T cells, IL-15 stimulates little proliferation of memory-phenotype $CD4^+$ T cells either *in vivo* or *in vitro*.[18] Furthermore, memory-phenotype $CD4^+$ T cells are found in normal numbers in IL-$15^{-/-}$ and IL-$15R\alpha^{-/-}$ mice.[168,169] Therefore, IL-15 does not appear to play a role in the maintenance of $CD4^+$ memory cells. In fact, $CD4^+$ memory cells are long-lived in mice deficient for the common γ-chain, indicating that none of the cytokines utilizing this receptor component (IL-2, IL-4, IL-7, IL-9 and IL-15) are essential for the survival of $CD4^+$ memory T cells.[71] Thus, if cytokines do play a role in the maintenance of $CD4^+$ memory T cells, the factors involved are clearly distinct from those that have effects on either naïve T cells or $CD8^+$ memory cells. Presently, the best evidence that the turnover of $CD4^+$ memory T cells can be regulated by cytokines comes from a study in which T-cell proliferation was assessed following the activation of natural killer ($NK1.1^+$) T cells *in vivo*.[172] Under these conditions, the proliferation of $CD44^{hi}$ $CD4^+$ ($NK1.1^-$) T cells was markedly increased through a mechanism that was dependent on IL-12 or IFN-γ.

CONCLUSIONS

There is considerable evidence to support the idea that T cells retain a permanent imprint of a prior response to antigen. In broad terms, naïve and memory T cells differ in phenotype, pattern of migration, responsiveness to antigen and cytokines and requirements for survival. However, it is also clear that memory T cells are heterogeneous with respect to all of these parameters. The recent identification of markers that can be used to separate subpopulations of memory T cells will allow a more precise delineation of the characteristics of memory cells.

Variation amongst memory T cells may arise as a result of the initial conditions of activation, but might also be influenced by the environment in which memory T cells reside. In this respect, future work aimed at elucidating the relationship between phenotypically distinct subpopulations of memory T cells should provide important insight into the nature of T-cell memory. For example, while it is evident that $CD4^+$ central memory cells can differentiate into cells resembling effector memory cells upon stimulation with antigen or cytokines *in vitro*, the rate at which this conversion takes place *in vivo*, and whether reversion from effector to central memory cell will occur in the absence of such stimulation, are not known.[17,173] In addition, the identification of memory T cells expressing certain markers that are characteristic of naïve T cells raises the question of whether some memory T cells may in fact be completely indistinguishable from naïve T cells in phenotype. If so, it would be of interest to determine whether these cells retain any functional characteristics of memory cells.

Finally, the extent to which the altered functional properties of memory T cells contribute to the characteristics of the secondary immune response remains unclear. In practice, changes at the single cell level probably synergize with increases in frequency in producing a rapid response to secondary infection (Fig. 1). Hence, effector memory T cells are able to respond immediately to re-infection on the basis of their distribution, reduced activation requirements and increased frequency, and should therefore limit the early spread of infection.[116]

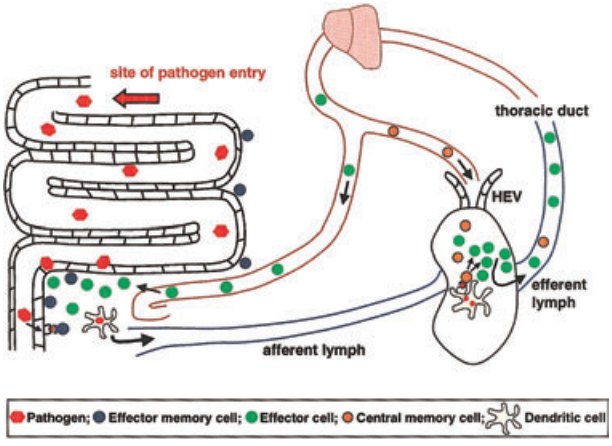

Figure 1. Combined effects of qualitative and quantitative changes in memory T cells in mediating a rapid secondary response to infection. Effector memory T cells are situated in peripheral tissues at sites of pathogen entry. These cells exhibit effector function immediately upon recognition of antigen presented on non-professional APCs and limit the early spread of infection. Central memory T cells are activated in secondary lymphoid organs (i.e. the draining lymph node) following recognition of antigen on DCs and generate large numbers of effector cells. The response of central memory cells is prompt as a result of both a high frequency of antigen specific cells and their ability to differentiate rapidly into effectors.

At the same time, central memory T cells are capable of generating rapidly a large number of effector cells based on their high precursor frequency; an accelerated ability to differentiate into effectors may also play a role.[174] These effector cells then distribute systemically and act to clear the residual infection. Understanding how memory T cells are maintained not only at a high frequency but also in a enhanced state of functional readiness therefore is important for both our fundamental understanding of the immune system and for the rational design of vaccines.

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